

The Effects of Seawater Flooding from Hurricane Sandy on the Diversity of Aquatic Invertebrate Microorganisms in Long Island Freshwater Ponds

By: Emily McGuire and Aileen Miller Mentor: Dr. Ellen McGlade-McCulloh
Mineola High School



Abstract

Freshwater ponds are usually rich in biodiversity, but may be altered by unpredictable weather conditions like Hurricane Sandy which has impacted many Long Island freshwater ponds. The increase of salt water could have negatively affected the flooded ponds, while the ponds not flooded would remain unaffected. If the freshwater ponds are flooded by Hurricane Sandy then the diversity of the microorganisms will be increased. DNA Barcoding was used to explain the differences in diversity between the two ponds. Samples were collected from Milburn Pond, where the waters were flooded, and Halls Pond, where the waters were not. The DNA was isolated, amplified through PCR and then put into gel electrophoresis for the researchers to find the bands. The samples were rejected initially since the DNA was not amplified enough. After repeating the procedure, three of the ten samples were successfully aligned and identified into species.

Introduction

Hurricane Sandy affected several American states along the eastern seaboard, as major flooding occurred along the coasts of Long Island. Temporary ponds tend to be rich in terms of biodiversity, but may be altered by unpredictable weather conditions or human interaction (Olmo, et al, 2012). Researchers in the Mediterranean found that the most recently restored ponds had the lowest richness of zooplankton because there was a limited amount of time for colonization and establishment in the egg bank (Olmo, et al, 2012). Other researchers found that smaller businesses are more susceptible to failure due to not being prepared just like the smaller plants not being able to spread to other places but microorganisms may be able to with the floodwaters (Hoggart, et al, 2014). Barcoding has allowed for researchers to be able to generate DNA sequences for all named species on the planet. Cytochrome c oxidase subunit I gene, COI, has been used to identify 1 billion different combinations of DNA (Herbert, et al, 2003). The COI system provides a less expensive and more reliable solution to identifying species, as it helps to avoid misidentifications. Scientists are hoping that it will explain the differences in populations of organisms, thus opening new doors in the world of science (Herbert, et al, 2003). The encroachment of saltwater on land could affect the availability of freshwater on Long Island and in it's aquifer. This study seeks to determine the effects of the seawater from such a massive storm on the diversity of microorganisms in freshwater ponds on Long Island. The hypothesis is that the freshwater ponds that have been flooded by Hurricane Sandy will contain more diversity than ponds that have not been flooded.

Materials & Methods

Consent was obtained from Deputy Commissioner Frank Camerlengo to collect samples at Milburn and Halls Pond Preserves in Nassau County. Milburn Pond, located in Baldwin, was flooded by Hurricane Sandy. Halls Pond, which was located in West Hempstead, was not flooded. Approximately one liter of water was extracted from each pond in November 2016. The samples were taken within 6 square inches of a nearby post. A smartphone was used to take wide, medium, and close-up shots of the sites as well as the latitude, longitude, and altitude coordinates. A thermometer was used to measure the temperature of the pond water. Five microorganisms were obtained and identified from each pond, making a total of ten microorganisms. These samples were stored in Eppendorf tubes and placed in a freezer until the DNA was isolated and amplified by polymerase chain reaction (PCR). The DNA was first added to tubes containing cytochrome c oxidase subunit I (COI) primer mix. The tubes were then placed in a thermal cycler with the appropriate PCR protocol. Gel electrophoresis was used to ensure that the amplification process was successful. Pictures were taken of the resulting bands and uploaded onto the Barcode sample database website in order to be approved for sequencing. Once the sequences were obtained, they were analyzed through DNA subway (www.dnasubway.org). The Barcode of Life Database was used to identify each species.

Results

Of the 10 samples that were collected within the study, only 3 were approved for sequencing. Samples PHW-101, PHW-102, and PHW-109 were sent to Cold Spring Harbor Laboratory for sequencing. Using blue line DNA Subway blue line, it was found that samples PHW-101 and PHW-102 were most closely aligned with Cypridopsis and sample PHW-109 was most closely aligned with Chydorus Brevilabris. Since samples PHW-101 and PHW-102 both have about 30 mismatches, they are potential novel sequences.

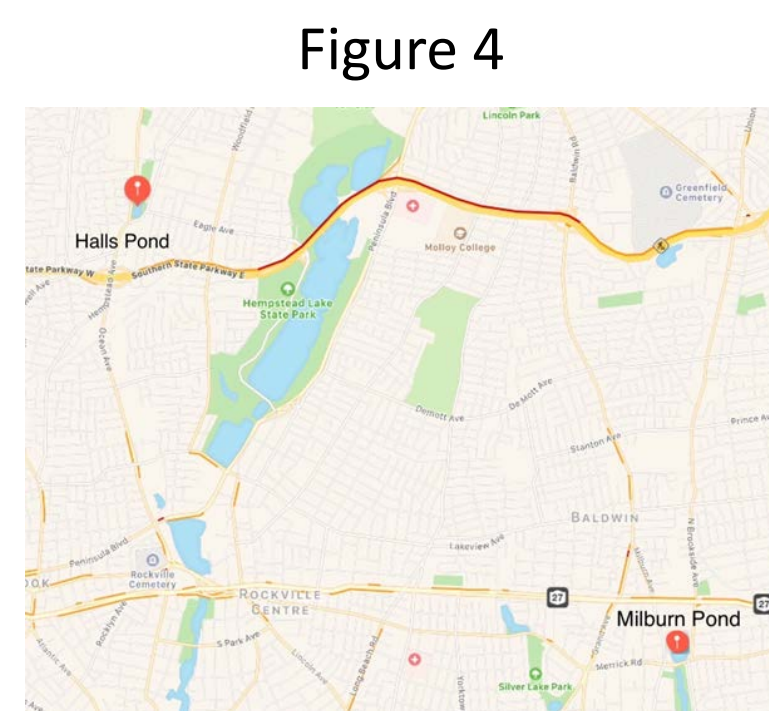


Figure 4: Map of the sites where samples were collected.

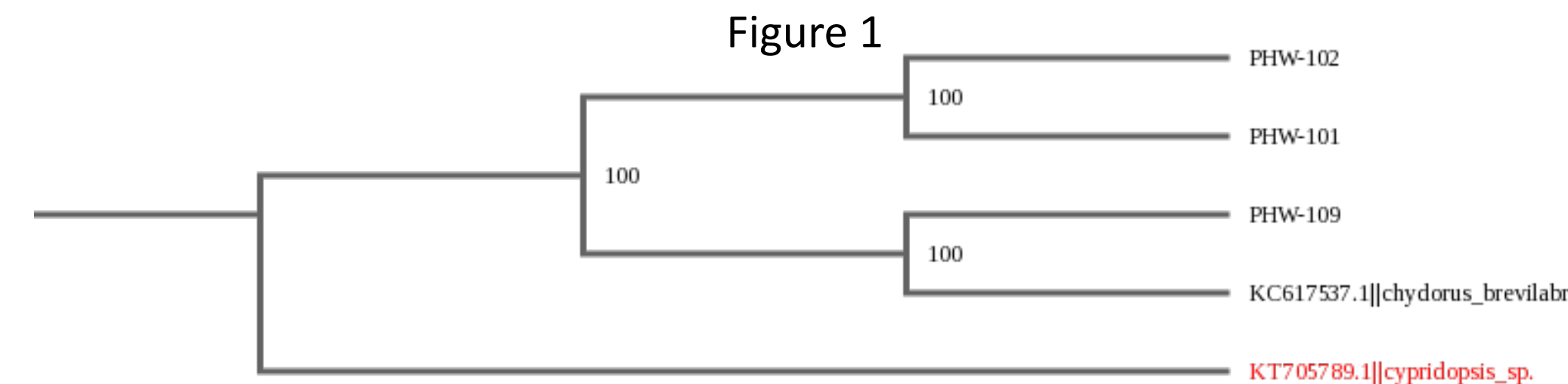


Figure 1: Phylogenetic tree. The figure shows the similarities between samples and species, aligning three of the samples to small invertebrates Cypridopsis and Chydorus.

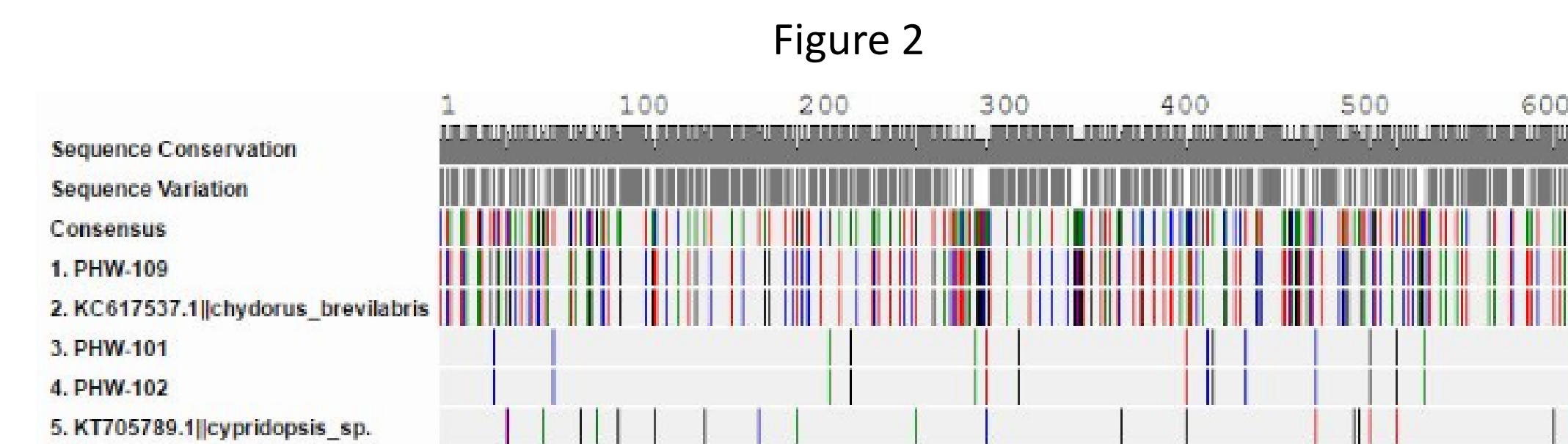
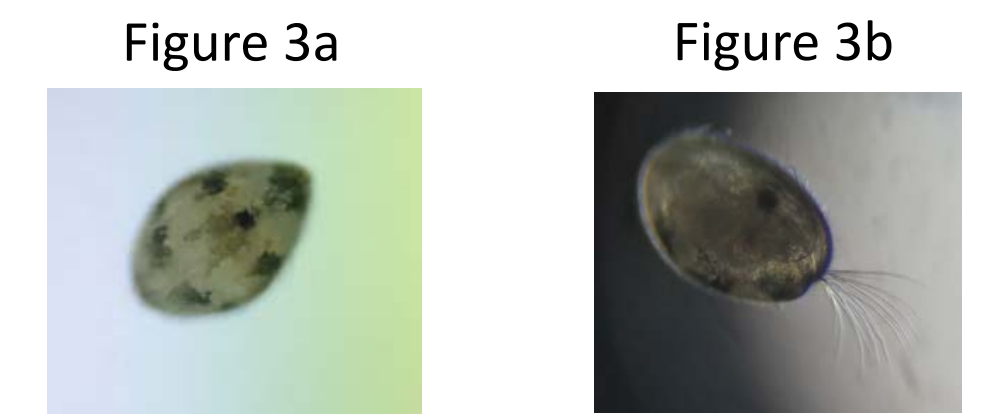
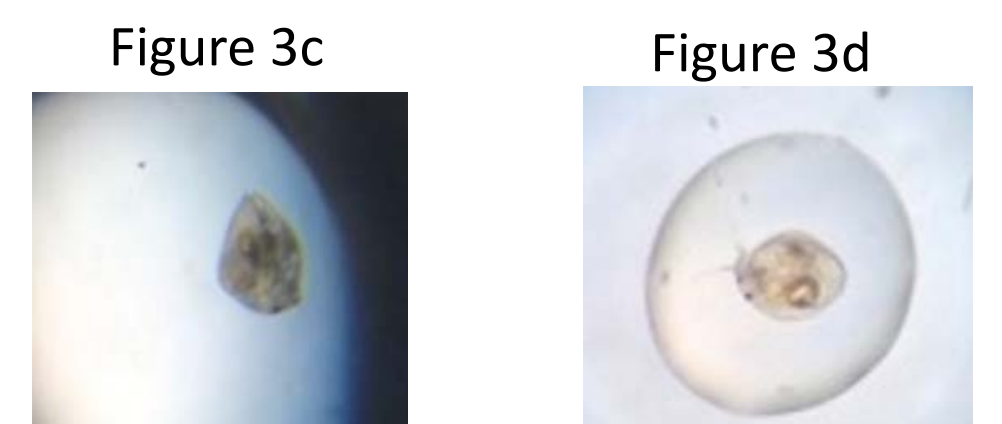


Figure 2: MUSCLE multiple alignment. This is a visual representation of the samples using DNA Subway blue line. It shows the alignment between the most closely related species from the BLAST hits and the samples collected. In the figure, the blue lines represent the cytosine nucleotide, green lines represent the adenine nucleotide, red lines represent the thymine nucleotide, and black lines represent the guanine nucleotide.

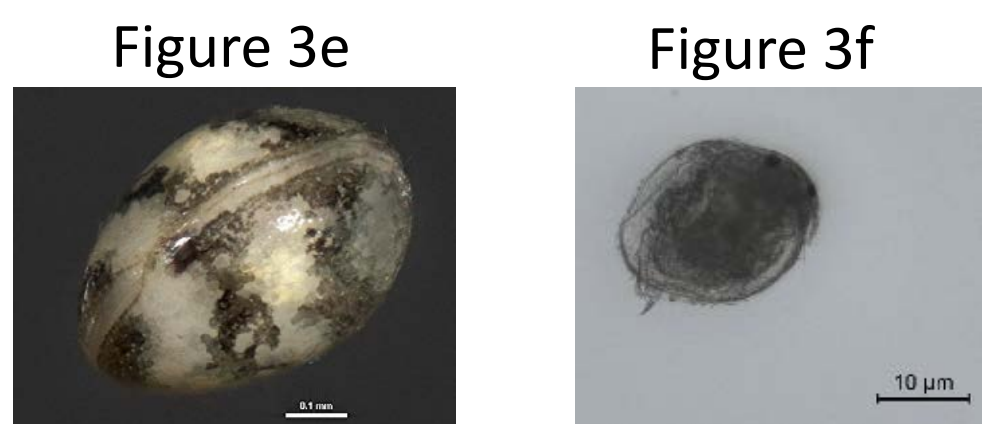
Figures 3e and 3f: Pictures of Cypridopsis and Chydorus Brevilabris species obtained from Barcode of Life Database



Figures 3a and 3b: The two samples shown, PHW-101 and PHW-102, were collected from Milburn Pond. Both of the microorganisms were successfully aligned and identified as the same species of Cypridopsis.



Figures 3c and 3d: The two samples shown, PHW-109 and PHW-110, were collected from Halls Pond. The DNA from PHW-110 was not able to be aligned, while PHW-109 was identified as Chydorus Brevilabris.



Discussion

Sequences PHW-101 to PHW-105 represented the samples from Milburn Pond, while sequences PHW-106 to PHW-110 represented samples from Halls Pond. It was found that samples PHW-101 and PHW-102 most closely resembled the species Cypridopsis, while sample PHW-109 most closely resembled the Chydorus Brevilabris. According to the alignment, samples PHW-101 and PHW-102 have the same sequences. The two microorganisms may be present in different life cycles, which could explain their differences in appearance. Because only three samples were successfully sequenced and identified as species, there is not enough information to effectively compare the diversity between the two freshwater ponds. The process should be repeated with new samples in order to get more sequences back. The samples should be grinded for a longer period of time and much less volume should be used.

References

[1] DeSalle, R., Egan, M. G., Siddall, M. (2005). The unholy trinity: taxonomy, species delimitation and DNA barcoding.
[2] Herbert, P. D. N., Cywinska, A., Ball, S. L., deWaard, J. R. (2003). Biological Identifications through DNA barcodes. Proc. R. Soc. Lond. 270:313-321.
[3] Hoggart, S.P.G., Hanley, M.E., Parker, D.J., Simmonds, D.J., Bilton, D.T., Filipova-Marino, M., Franklin, E.L., Kotsev, I., Penning-Rowsell, E.C., Rundle, S.D., Trifonova, E., Vergiev, S., White, A.C., Thompson, R.C. (2014). The consequences of doing nothing: The effects of seawater on coastal zones. Coastal Engineering. 87:169-182.
[4] Lear, G., Bellamy, J., Case, B.S., Lee, J.E., Buckley, H.L. (2014) Fine-scale spatial patterns in bacterial community composition and function within freshwater ponds. The ISME Journal. 8: 1715-1726
[5] Mhuantong, W., Wongwilaiwalin, S., Laothanachareon, T., Eurwilachit, L., Tangphatsornruang, S., Boonchayaanant, B., Limpiyakorn, T., Pattaragulwanit, K., Punmatharith, T., McEvoy, J., Khan, E., Rachakornkij, M., Champredal, V. (2015) Survey of Microbial Diversity in Flood Areas during Thailand 2011 Flood Crisis Using High- Throughput Tagged Amplicon Pyrosequencing. PLoS ONE 10(5): e0128043.
[6] Olmo, C., Armengol, X., Ortells, R. (2012). Re-establishment of zooplankton communities in temporary ponds after autumn flooding: Does restoration age matter? Limnologia. 42:310-319.
[7] Unger, I. M., Kennedy, A. C., Muzika, R. (2009). Flooding effects on soil microbial communities. Applied Soil Ecology. 42:1-8.
[8] Wang, Y., Huang, Y., Qiu, Q., Xin, G., Yang, Z., Shi, S. (2011). Flooding Greatly Affects the Diversity of Arbuscular Mycorrhizal Fungi Communities in the Roots of Wetland Plants. PLoS ONE 6(9): e24512

Acknowledgements

We would like to thank the staff at Cold Spring Harbor Laboratory for their assistance, especially Cristina Fernandez-Marco.